Actinokineospora spheciospongiae sp. nov., isolated from the marine sponge *Spheciospongia vagabunda*

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A Gram-staining-positive, aerobic organism, isolated from the Red Sea sponge *Spheciospongia vagabunda* was investigated to determine its taxonomic position. On the basis of results of 16S rRNA gene sequence analysis strain EG49^T was most closely related to *Actinokineospora cibodasensis* and *Actinokineospora baliensis* (both 97.3 % similarity) and *Actinokineospora diospyrosa* and *Actinokineospora auranticolor* (both 97.0 % similarity). The 16S rRNA gene sequence similarity to all other species of the genus *Actinokineospora* was <97.0 %. The quinone system of strain EG49^T contained the menaquinones MK-9(H_4) (47 %), MK-9(H_6) (27 %) and MK-9(H_2) (15 %) in major amounts. Minor amounts of MK-7(H_4) (2 %), MK-9(H_0) (1 %), MK-9(H_8) (3 %) and MK-10(H_4) (3 %) were detected as well in addition to MK-8(H_4), MK-8(H_6), MK-10(H_2) and MK-10(H_6) (all <1 %). The diagnostic diamino acid of the peptidoglycan was *meso*-diaminopimelic acid. In the polar lipid profile, diphosphatidylglycerol, phosphatidylethanolamine and hydroxyphosphatidylethanolamine were predominant. Phosphatidylinositol-mannoside, two unidentified phospholipids and two glycoglipids as well as one aminoglycolipid, one aminolipid and one unidentified lipid were found in addition. The fatty acid profile was composed of mainly iso-branched fatty acids: iso-$C_{16:0}$, iso-$C_{14:0}$, iso-$C_{15:0}$ and iso-$C_{16:1}$H. All these findings clearly supported the classification of the strain as representing a member of the genus *Actinokineospora*. In addition, the results of physiological and biochemical tests also allowed phenotypic differentiation of strain EG49^T from the most closely related species of the genus *Actinokineospora*. Strain EG49^T represents a novel species of the genus *Actinokineospora*, for which we propose the name *Actinokineospora spheciospongiae* sp. nov., with strain EG49^T (=DSM 45935^T=CCM 8480^T=LMG 27700^T) as the type strain.

The genus *Actinokineospora* was proposed by Hasegawa (1988), initially for motile, arthrospore-producing organisms of the class *Actinomycetes*. Labeda *et al.* (2010) recently emended the description of the genus, which now harbours also species for which the production of motile spores was not observed, and proposed the transfer of *Amycolatopsis fastidiosa* to this genus as *Actinokineospora fastidiosa*. The genus contains, at the time of writing, 13 species: *Actinokineospora riparia* (the type species), *Actinokineospora inagensis*, *Actinokineospora globicatena*, *Actinokineospora terrae*, *Actinokineospora diospyrosa*, *Actinokineospora auranticolor*, *Actinokineospora enzanensis*, *Actinokineospora fastidiosa*, *Actinokineospora baliensis*, *Actinokineospora bangkokensis*, *Actinokineospora cianjurensis*, *Actinokineospora cibodasensis* and *Actinokineospora soli* (Hasegawa, 1988; Tamura *et al.*, 1995; Otoguro *et al.*, 2001; Labeda *et al.*, 2010, Lisdiyanti *et al.*, 2010; Tang *et al.*, 2012; Intra *et al.*, 2013). These actinomycetes are characterized by having *meso*-diaminopimelic acid as a cell-wall diamino acid, MK-9(H_4) as the predominant

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menaquinone, phospholipid type II, iso-$C_{16:0}$ fatty acid as the predominant fatty acid and DNA G+C contents of 69–74 mol%.

Strain EG49^T was isolated by Abdelmohsen *et al.* (2010) from the marine sponge *Spheciospongia vagabunda* collected from the Red Sea (Ras Mohamed, Sinai, Egypt; GPS coordinates 27° 47.655′ N 34° 12.904′ W). The metabolomic and the genomic analyses of the strain showed its richness with diverse bioactive natural products. (Abdelmohsen *et al.*, 2014; Harjes *et al.*, 2014; Grkovic *et al.*, 2014).

The strain showed the presence of aerial mycelium with spore chains. The spores were rod-shaped and were formed by fragmentation of the hyphae (arthrospores). Cultural characteristics were recorded after 14 days of incubation at 28 °C on International Streptomyces Project (ISP) 2 medium, tryptone soy agar (TSA; Oxoid)) and nutrient agar (Oxoid). Light yellow to brown colonies were produced on these media. The isolate exhibited good growth on all of the media.

Cells displayed Gram-positive staining (analysed as described by Gerhardt *et al.*, 1994) and were negative for cytochrome oxidase, determined by using an oxidase test (Merck). Endospores could not be detected. Temperature-dependent growth was determined on ISP2 agar at 4, 15, 25, 28, 32, 37 and 42 °C. Salinity- and pH-dependent growth were analysed in ISP2 broth either supplemented with 1–10 % (w/v) NaCl or adjusted to pH values between pH 4 and 12 (at intervals of 0.5 pH units, adjusted by the addition of HCl or NaOH) and cultured at 28 °C.

Detailed phylogenetic analysis based on 16S rRNA gene sequences was performed in ARB release 5.2 (Ludwig *et al.*, 2004) using the 'All-Species Living Tree' Project (LTP) (Yarza *et al.*, 2008) database LTPs115 (March, 2014). The 16S rRNA gene sequence of strain EG49^T was aligned with the SILVA Incremental Aligner (SINA; v1.2.11; Pruesse *et al.*, 2012) according to the SILVA seed alignment [http://www.arb-silva.de; Pruesse *et al.* (2007)]. The aligned sequence was implemented into the LTP database and added to the database tree using the ARB Parsimony (Quick add marked) tool. The alignment, including all members of the family *Pseudonocardiaceae* and some outgroup species of the family *Micromonosporaceae*, was checked manually before reconstruction of phylogenetic trees. A maximum-likelihood tree was reconstructed using RAxML v7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis. The tree was based on 16S rRNA gene sequence termini 73–1443 (*Escherichia coli* numbering; Brosius *et al.*, 1978). The phylogenetic tree showed a clear allocation of strain EG49^T, clustering among species of the genus *Actinokineospora*. Based on these results, further phylogenetic analyses were performed including all species of the genus *Actinokineospora* and species of the genus *Kutzneria* as the outgroup. Again, a maximum-likelihood tree, a neighbour-joining tree using ARB neighbour-joining and the Jukes–Cantor correction (Jukes & Cantor, 1969) and a maximum-parsimony tree using DNAPARS v 3.6 (Felsenstein, 2005) were generated. The phylogenetic trees were reconstructed with 100 resamplings (bootstrap analysis; Felsenstein, 1985) and based on 16S rRNA gene sequence termini 62–1459 (*E. coli* numbering, Brosius *et al.*, 1978). Pairwise sequence similarities among the type strains of species of the genus *Actinokineospora* were calculated using the ARB neighbour-joining tool without the use of an evolutionary substitution model.

The sequenced 16S rRNA gene of strain EG49^T represents a continuous stretch of 1481 nt spanning *E. coli* positions 9–1514 (*E. coli* numbering; Brosius *et al.*, 1978). Strain EG49^T shared highest 16S rRNA gene sequence similarity with the type strains of *Actinokineospora cibodasensis* and

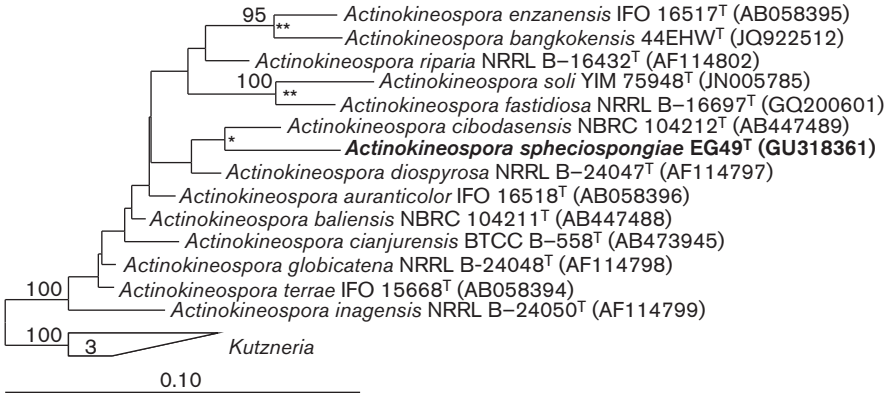

Fig. 1. Maximum-likelihood tree showing the phylogenetic position of strain EG49^T among type strains of species of the genus *Actinokineospora*. The tree was generated in ARB using RAxML (GTR-GAMMA, Rapid Bootstrap analysis) and based on nucleotide sequences at 16S rRNA gene sequence positions 62–1459 (*E. coli* numbering). Type strains of species of the genus *Kutzneria* were used as the outgroup. Bootstrap values ⩾70 % are given in the tree. Nodes marked with asterisks also occurred in the maximum-parsimony and neighbour-joining trees; those nodes marked with two asterisks also showed bootstrap values >70 %. Bar, 0.1 substitutions per nucleotide position.

Actinokineospora baliensis (both 97.3 %) and *Actinokineospora diospyrosa* and *Actinokineospora auranticolor* (both 97.1 %). The 16S rRNA gene sequence similarities to all other type strains of species of the genus *Actinokineospora* were below 97.0 %. Results of phylogenetic analysis showed that strain EG49^T clustered within the genus *Actinokineospora* with the type strain of *Actinokineospora cibodasensis*. The clustering was obtained with all applied treeing methods, but was not supported by high bootstrap values, indicating that the direct phylogenetic relationship of the novel strain and the species of the genus *Actinokineospora* cannot be resolved at the species level solely on the basis of 16S rRNA gene sequence analysis (Fig. 1). Because of the low 16S rRNA gene sequence similarities to all species of the genus *Actinokineospora* (<97.3 %), DNA–DNA hybridizations were not performed. In none of the previously published studies, DNA–DNA pairing values >50 % could be found for pairs of strains showing <98 % 16S rRNA gene sequence similarity (Tamura *et al.*, 1995; Lisdiyanti *et al.*, 2010; Tang *et al.*, 2012; Intra *et al.*, 2013). Furthermore, Meier-Kolthoff *et al.* (2013) showed clearly for the class *Actinobacteria* that at a threshold of 97.6 % 16S rRNA gene sequence similarity between a pair of strains, the maximum probability of an error that these strains represent the same species is only 0.01 %.

Biomass subjected to analyses of diamino acids, quinones and polar lipids was grown in PYE broth (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) at 28 °C. Biomasses used for extraction of diamino acids, quinones and polar lipids were harvested at the stationary growth phase. Diamino acid extraction was carried out according to the protocol of Schumann (2011). Quinones and polar lipids were extracted and analysed as described by Tindall (1990a, b) and Altenburger *et al.* (1996). The HPLC apparatus used was described by Stolz *et al.* (2007). The diagnostic diamino acid of the peptidoglycan was *meso*-diaminopimelic acid. Strain EG49^T showed a complex quinone system which contained 47 % MK-9(H_4), 27 % MK-9(H_6) and 15 % MK-9(H_2). Minor amounts of MK-7(H_4) (2 %), MK-9(H_0) (1 %), MK-9(H_8) (3 %) and MK-10(H_4) (3 %) were detected as well in addition to MK-8(H_4), MK-8(H_6), MK-10(H_2) and MK-10(H_6) (all <1 %).

The polar lipid profile (Fig. 2) consisted of the major lipids diphosphatidylglycerol, phosphatidylethanolamine and hydroxyphosphatidylethanolamine. Phosphatidylinositol mannoside, two unidentified phospholipids and two glycoglipids as well as one aminoglycolipid, one aminolipid and one unidentified lipid were found in addition.

The *meso*-diaminopimpelic acid detected in the peptidoglycan and the quinone system consisting predominantly of menaquinone MK-9(H_4) is in agreement with the description of the genus *Actinokineospora*. The polar lipid profile

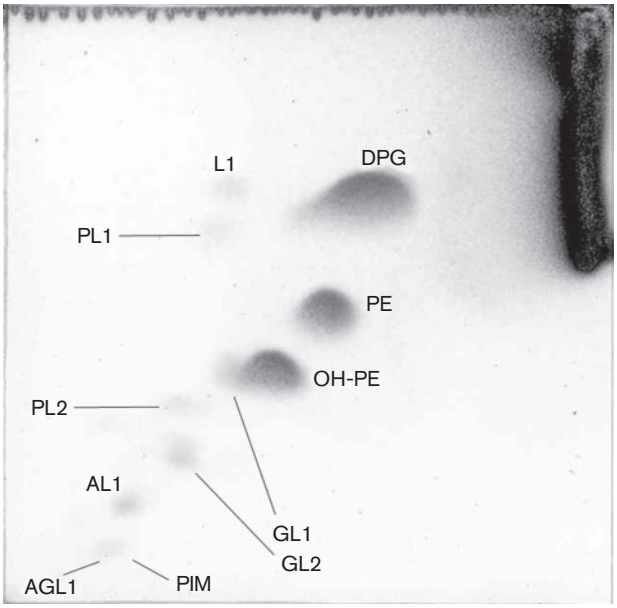

Fig. 2. Polar lipid profile of strain EG49^T after two-dimensional TLC and detection with molybdatophosphoric acid. DPG, diphosphatidylglycerol; OH-PE, hydroxyphosphatidylathanolamine; PE, phosphatidylethanolamine; PIM, phosphatidylinositol mannoside; GL1, GL2, unidentified glycolipids; AL1, unidentified aminolipid; AGL1, unidentified amnioglycolipid; PL1, PL2, unidentified phospholipids; L1, unidentified polar lipid.

Table 1. Major fatty acid composition of strain EG49^T and the type strains of the most closely related species

Strains: 1, EG49^T; 2, *Actinokineospora diospyrosa* NRRL B-24047^T; 3, *Actinokineospora riparia* NRRL B-16432^T; 4, *Actinokineospora auranticolor* CIP 107896^T; 5, *Actinokineospora baliensis* NBRC 104211^T; 6, *Actinokineospora cibodasensis* NBRC 104212^T. All data from this study obtained from cells grown on TSA after 3 days at 28 °C.

Fatty acid	1	2	3	4	5	6
iso-$C_{14:0}$	8.7	5.5	11.3	12.2	12.6	1.6
iso-$C_{14:0}$ 3-OH			3.4	3.9		
$C_{15:1}\omega6c$					3.1	2.0
iso-$C_{15:0}$	7.8	25.2	13.8	13.7	19.7	18.2
anteiso-$C_{15:0}$		4.9	2.2	2.0	2.7	1.4
$C_{15:0}$			4.5	4.4	3.3	3.3
iso-$C_{16:1}$ H	10.7	8.3	4.2	3.1	5.8	2.7
$C_{16:1}$ 2-OH				1.4		
iso-$C_{16:0}$	59.3	39.7	47.5	48.8	35.8	24.9
$C_{16:0}$			1.5		1.7	3.1
Summed feature 3*	6.9		3.5	1.7	4.9	7.2
iso-$C_{17:1}\omega9c$						1.2
$C_{17:1}\omega6c$			6.4	2.9	2.1	4.5
$C_{17:1}\omega8c$					3.3	10.2
iso-$C_{17:0}$		8.1		1.7	2.0	6.2
anteiso-$C_{17:0}$		8.3	1.8	2.2		9.7
$C_{17:0}$ cyclo	6.6					
$C_{17:0}$				1.7	3.0	3.7

*Summed feature 3 comprises $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

Table 2. Differential phenotypic characteristics of EG49^T and the type strains of the closest related species

Strains: 1, EG49^T; 2, *Actinokineospora diospyrosa* NRRL B-24047^T; 3, *Actinokineospora riparia* NRRL B-16432^T; 4, *Actinokineospora auranticolor* CIP 107896^T; 5, *Actinokineospora baliensis* NBRC 104211^T; 6, *Actinokineospora cibodasensis* NBRC 104212^T; 7, *Alloactinosynnema album* CCM 7461^T. All data are from this study. +, Positive; −, negative; (+), weakly positive. All strains were negative for acid production from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, *myo*-inositol, L-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, D-arabitol and D-mannose. None of the strains hydrolysed *para*-nitrophenyl (pNP)-β-D-glucuronide and pNP-β-D-xylopyranoside and none of the strains assimilated L-arabinose, *p*-arbutin, α-melibiose, salicin, adonitol, *myo*-inositol, D-sorbitol, putrescine, *trans*-aconitate, 4-aminobutyrate, citrate, itaconate, mesaconate, 3-hydroxybenzoate or 4-hydroxybenzoate as a sole source of carbon. All strains were positive for hydrolysis of L-alanine-pNA and assimilation of *N*-acetyl-D-glucosamine, D-glucose and oxoglutarate as sole sources of carbon.

Characteristic	1	2	3	4	5	6	7
Hydrolysis of:							
Aesculin	+	−	(+)	(+)	(+)	+	+
oNP-β-D-galactopyranoside	−	−	−	−	−	−	+
pNP-α-D-glucopyranoside	+	+	+	−	+	+	+
pNP-β-D-glucopyranoside	+	−	(+)	−	−	+	(+)
Bis-pNP-phosphate	−	+	+	+	+	+	+
pNP-phenylphosphonate	−	(+)	+	+	+	+	+
pNP-phosphorylcholine	−	−	+	−	−	−	+
2-Deoxythymidine-5′-thymidine-pNP-phosphate	+	+	+	−	+	+	+
L-Glutamate-γ-3-carboxy-*para*-nitroanilide	+	(+)	(+)	−	+	+	+
L-Proline-pNA	+	+	−	+	+	+	+
Assimilation of:							
N-Acetyl-D-galactosamine	−	−	−	−	−	−	+
Cellobiose	+	−	−	−	−	−	+
D-Fructose	+	(+)	−	−	−	+	+
D-Galactose	−	−	−	−	−	−	+
Gluconate	+	+	+	+	+	+	−
D-Glucose	(+)	+	+	+	+	+	+
Maltose	+	(+)	−	−	−	−	+
D-Mannose	+	(+)	−	−	−	+	+
L-Rhamnose	(+)	−	−	−	−	−	−
D-Ribose	−	−	−	−	−	+	−
Sucrose	+	+	−	−	−	(+)	+
Salicin	−	−	−	−	−	−	−
Trehalose	+	+	(+)	+	−	−	+
D-Xylose	+	−	−	−	−	−	−
Maltitol	(+)	−	−	−	−	−	−
D-Mannitol	+	−	−	−	−	−	+
Acetate	+	(+)	(+)	−	+	+	+
Propionate	+	−	−	−	+	+	(+)
cis-Aconitate	−	−	−	−	−	−	+
Adipate	(+)	−	−	−	−	−	−
Azelate	(+)	−	−	−	−	−	+
Fumarate	(+)	−	(+)	+	−	+	+
Glutarate	(+)	−	(+)	−	−	−	−
DL-3-Hydroxybutyrate	−	−	−	−	(+)	−	−
DL-Lactate	−	−	−	−	−	(+)	−
L-Malate	(+)	−	−	+	−	+	+
Pyruvate	(+)	−	−	+	−	+	+
Suberate	+	(+)	−	−	−	−	+
L-Alanine	+	+	−	+	(+)	+	+
β-Alanine	−	−	−	(+)	(+)	−	−
L-Aspartate	(+)	+	(+)	(+)	+	−	+
L-Histidine	+	+	−	(+)	+	+	+
L-Leucine	(+)	−	−	−	(+)	+	+
L-Ornithine	+	(+)	−	−	(+)	+	+
L-Phenylalanine	(+)	−	−	(+)	+	+	+

Table 2. cont.

Characteristic	1	2	3	4	5	6	7
L-Proline	(+)	+	−	+	+	+	+
L-Serine	(+)	+	−	+	(+)	+	+
L-Tryptophan	(+)	(+)	−	−	−	+	(+)
Phenylacetate	(+)	−	−	−	−	−	−

of strain EG49^T showed some similarities to those of other species of the genus *Actinokineospora* with respect to the presence of phospholipids, but showed also some differences.

Fatty acids analysis of cells, grown in tryptone soy broth (TSB) at 28 °C, was done as described by Kämpfer & Kroppenstedt (1996) using the Sherlock Microbial Identification System (Sherlock software version 2.11 and a TSBA peak naming table version 4.1; MIDI).

The fatty acid profile comprised mainly iso-branched fatty acids and was similar to those of the most closely related species (Table 1).

The results of the physiological characterization, performed using methods described previously (Kämpfer *et al.*, 1991), are given in Table 2 and in the species description. Strain EG49^T was able to utilize several sugars or sugar-related compounds. A distinct physiological and biochemical profile allowed differentiation of the strain from the type strains of the most closely related species of the genus *Actinokineospora*. Based on the low 16S rRNA gene sequence similarities (<97.3 %) to all other species of the genus with validly published names, DNA–DNA hybridizations were not performed. From the results of the phylogenetic and chemotaxonomic analyses, it is obvious that strain EG49^T represents a novel species, which is allocated to the genus *Actinokineospora*. For this species we propose the name *Actinokineospora spheciospongiae* sp. nov.

Description of *Actinokineospora spheciospongiae* sp. nov.

Actinokineospora spheciospongiae (sphe.ci.o.spon′gi.ae N.L. gen. n. *spheciospongia* of/from *Spheciospongia* the zoological name of a genus of sponge, referring to the isolation of the type strain from the sponge *Spheciospongia vagabunda*).

Vegetative mycelium is yellow to tan. When formed, aerial mycelium is white. Aerial mycelium produces rod-shaped arthrospores (diameter 1.5–1.8 μm). Motility of spores is not observed. Good growth is observed at 25–28 °C. Grows well on ISP media 2 and 3 and on tryptone soy agar and nutrient agar. Optimal temperature for growth is 28 °C; growth occurs at 20–36 °C but not at 15 °C and below or 40 °C and above on ISP2 agar. Optimal pH for growth is pH 7.0; growth occurs at pH 5.5–10.5. Growth is observed with a NaCl concentration from 1 % (w/v) up to 5 % (w/v), but not above. Test for catalase is positive; oxidase activity is weakly positive. No acid formation can be observed from D-glucose, D-xylose, lactose, sucrose, D-mannitol, dulcitol, salicin, D-adonitol, *myo*-inositol, D-sorbitol, L-arabinose, raffinose, L-rhamnose, maltose, trehalose, cellobiose, erythritol, melibiose or D-arabitol. Several sugar compounds are utilized including *N*-acetyl-D-glucosamine, D-fructose, cellobiose, D-gluconate, D-glucose, D-mannose, maltose, D-maltitol (weakly), D-mannitol, L-rhamnose (weakly), sucrose, trehalose and D-xylose. L-Arabinose, arbutin, D-galactose, D-adonitol, *myo*-inositol, melibiose, ribose, D-sorbitol and salicin are not utilized. Major fatty acids are iso-$C_{16:0}$, iso-$C_{14:0}$, iso-$C_{15:0}$ and iso-$C_{16:1}$ H. The diagnostic diamino acid of the peptidoglycan is *meso*-diaminopimelic acid. Major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and hydroxyphosphatidylethanolamine. Phosphatidylinositol mannoside, two unidentified phospholipids and two glycoglipids as well as one aminoglycolipid, one aminolipid and one unidentified lipid are detected in addition. Major quinones are MK-9(H_4), MK-9(H_6) and MK-9(H_2). Minor amounts of MK-7(H_4), MK-9(H_0), MK-9(H_8) and MK-10(H_4) are detected as well in addition to MK-8(H_4), MK-8(H_6), MK-10(H_2) and MK-10(H_6).

The type strain, EG49^T (=DSM 45935^T=CCM 8480^T=LMG 27700^T), was isolated on ISP 2 medium from the Red Sea sponge *Spheciospongia vagabunda*.

References

Abdelmohsen, U. R., Cheng, C., Viegelmann, C., Zhang, T., Grkovic, T., Quinn, R. J., Safwat A., Hentschel, U. & Edrada-Ebel, R. (2014). Dereplication strategies for targeted isolation of new anti-trypanosomal actinosporins A and B from a marine sponge associated-*Actinokineospora* sp. EG49. *Mar Drugs* **12**, 1220–1244.

Abdelmohsen, U. R., Pimentel-Elardo, S. M., Hanora, A., Radwan, M., Abou-El-Ela, S. H., Ahmed, S. & Hentschel, U. (2010). Isolation, phylogenetic analysis and anti-infective activity screening of marine sponge-associated actinomycetes. *Mar Drugs* **8**, 399–412.

Altenburger, P., Kämpfer, P., Makristathis, A., Lubitz, W. & Busse, H.-J. (1996). Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* **47**, 39–52.

Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.

Felsenstein, J. (1985). Confidence limits of phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Felsenstein, J. (2005). PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.

Grkovic, T., Abdelmohsen, U. R., Othman, E. M., Stopper, H., Edrada-Ebel, R., Hentschel, U. & Quinn R. J. (2014). Two new Antioxidant actinosporin analogues from the calcium alginate beads culture of sponge-associated *Actinokineospora* sp. strain EG49. *Bioorg Med Chem Lett* **24**, 5089–5092.

Harjes, J., Ryu, T., Abdelmohsen, U. R., Moitinho-Silva, L., Horn, H., Ravasi, T. & Hentschel, U. (2014). Draft genome sequence of the antitrypanosomally active sponge associated-bacterium *Actinokineospora* sp. strain EG49. *Genome Announcments*. Doi:10.1128/genomeA.00160-14.

Hasegawa, T. (1988). *Actinokineospora*: a new genus of the actinomycetales. *Actinomycetologica* **2**, 31–45.

Intra, B., Matsumoto, A., Inahashi, Y., Omura, S., Takahashi, Y. & Panbangred, W. (2013). *Actinokineospora bangkokensis* sp. nov., isolated from rhizospheric soil. *Int J Syst Evol Microbiol* **63**, 2655–2660.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of the protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.

Kämpfer, P., Steiof, M. & Dott, W. (1991). Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227–251.

Labeda, D. P., Price, N. P., Tan, G. Y. A., Goodfellow, M. & Klenk, H.-P. (2010). Emended description of the genus *Actinokineospora* Hasegawa 1988 and transfer of *Amycolatopsis fastidiosa* Henssen *et al.* 1987 as *Actinokineospora fastidiosa* comb. nov. *Int J Syst Evol Microbiol* **60**, 1444–1449.

Lisdiyanti, P., Otoguro, M., Ratnakomala, S., Lestari, Y., Hastuti, R. D., Triana, E., Katsuhiko, A. & Widyastuti, Y. (2010). *Actinokineospora baliensis* sp. nov., *Actinokineospora cibodasensis* sp. nov. and *Actinokineospora cianjurensis* sp. nov., isolated from soil and plant litter. *Int J Syst Evol Microbiol* **60**, 2331–2335.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.

Meier-Kolthoff, J. P., Göker, M., Spröer, C. & Klenk, H.-P. (2013). When should a DDH experiment be mandatory in microbial taxonomy? *Arch Microbiol* **195**, 413–418.

Otoguro, M., Hayakawa, M., Yamazaki, T., Tamura, T., Hatano, K. & Iimura, Y. (2001). Numerical phenetic and phylogenetic analysis of *Actinokineospora* isolates, with a description of *Actinokineospora auranticolor* sp. nov. and *Actinokineospora enzaensis* sp. nov. *Actinomycetologica* **15**, 30–39.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J. & Glöckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* **35**, 7188–7196.

Pruesse, E., Peplies, J. & Glöckner, F. O. (2012). SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* **28**, 1823–1829.

Schumann, P. (2011). Peptidoglycan structure. *Methods Microbiol* **38**, 101–129.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.

Stolz, A., Busse, H.-J. & Kämpfer, P. (2007). *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* **57**, 572–576.

Tamura, T., Hayakawa, M., Nonomura, H., Yokota, A. & Hatano, K. (1995). Four new species of the genus *Actinokineospora: Actinokineospora inagensis* sp. nov., *Actinokineospora globicatena* sp. nov., *Actinokineospora terrae* sp. nov. and *Actinokineospora diospyrosa* sp. nov. *Int J Syst Evol Microbiol* **45**, 371–378.

Tang, X., Zhou, Y., Zhang, J., Ming, H., Nie, G.-X., Yang, L.-L., Tang, S.-K. & Li, W.-J. (2012). *Actinokineospora soli* sp. nov., a thermotolerant actinomycete isolated from soil, and emended description of the genus *Actinokineospora*. *Int J Syst Evol Microbiol* **62**, 1845–1849.

Tindall, B. J. (1990a). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Tindall, B. J. (1990b). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.

Yarza, P., Richter, M., Peplies, J., Euzeby, J., Amann, R., Schleifer, K. H., Ludwig, W., Glöckner, F. O. & Rosselló-Móra, R. (2008). The All-Species Living Tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. *Syst Appl Microbiol* **31**, 241–250.